AMENDMENT & REQUEST FOR CONTINUED EXAMINATION

Serial No.: 09/464,158 Filed: December 16, 1999

Title: ASSAY FOR CARBOHYDRATE-FREE TRANSFERRIN



- 1. (Amended) A method for the determination of carbohydrate-free transferrin in a body fluid for use in the assessment of elevated alcohol consumption, said method comprising
- (a) contacting a sample of said body fluid with a carbohydrate-binding ligand, to bind any carbohydrate or carbohydrate-containing moieties in said sample to said ligand;
- (b) separating a carbohydrate-free transferrin containing fraction not binding to said ligand and contacting the separated fraction with an anti-transferrin antibody or an anti-transferrin antibody fragment; and
- (c) determining the content of <u>carbohydrate-free</u> transferrin in said fraction and thereby determining the content of carbohydrate-free transferrin in said sample.
- 2. A method as claimed in claim 1, wherein the sample is blood or obtained from blood.
- 3. (Amended) [A] <u>The</u> method as claimed in claim 1, wherein the carbohydrate-binding ligand is selected from <u>the group consisting of</u> antibodies, [or] antibody fragments [thereof], lectins, [and] mammalian [or] <u>carbohydrate-binding proteins</u>, microbial carbohydrate-binding proteins, and mixtures thereof.
- 4. (Amended) [A] <u>The</u> method as claimed in claim 1, wherein in step (a) a panel of more than one type of lectin is used <u>as a carbohydrate binding ligand</u>.
- 5. (Amended) [A] <u>The</u> method as claimed in claim 1, wherein the carbohydrate_binding ligand is selected from the group consisting of *Sambucus nigra* lectin, *Sambucus sielbodiana* lectin, wheatgerm agglutinin, *Maackia amurensis* lectin, *E. coli* K99 lectin, *Helicobacter pylori* lectin, *Ricinus communis* lectin, [and] *Crotalaria junctae* lectin, [and] anti-sialic acid antibodies, and mixtures thereof.



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6. (Amended) [A] <u>The</u> method as claimed in claim 1, wherein the separation step (b) is by precipitation, centrifugation, filtration or chromatographic methods.

- 7. (Amended) [A] <u>The</u> method as claimed in claim 1, wherein the carbohydrate-binding ligand is immobilized.
- 8. (Amended) [A] <u>The</u> method as claimed in claim 1, wherein an ion exchange step to remove or deplete carbohydrate-carrying transferrins in the sample is performed prior to step (a).
- 9. (Amended) [A] <u>The</u> method as claimed in claim 1, wherein [the determination of] <u>determining the</u> transferrin content in step (c) is achieved by turbidometric or nephelometric means.



10. (Amended) A kit for use in a method as defined in claim 1, said kit comprising:

one or more carbohydrate-binding ligands;

means for separating unbound carbohydrate-free transferrin from ligandbound carbohydrate-containing transferrin; and

means for [the detection of transferrin] <u>determining the carbohydrate-free</u> <u>transferrin content in the separated portion which determines the content of carbohydrate-free transferrin in the sample</u>.

- 11. (Amended) [A] <u>The</u> kit as claimed in claim 10, wherein said means for [detection of transferrin comprise] <u>determining the carbohydrate-free transferrin</u> content comprises an anti-transferrin antibody or <u>an anti-transferrin</u> antibody fragment; and [preferably,] <u>optionally</u> an opacification enhancer.
- 12. (Amended) [A] <u>The</u> kit as claimed in claim 10, further comprising a <u>carbohydrate-free</u> transferrin solution of known concentration or a set of such solutions having a range of <u>carbohydrate-free</u> transferrin concentrations.

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Please add the following new claims 13-17.

- 13. (New) A method for the detecting carbohydrate-free transferrin in a body fluid for use as an indicator of alcohol abuse, said method comprising
- (a) contacting a sample of said body fluid with an immobilized carbohydrate-binding ligand to bind any carbohydrate-containing moieties in the sample to the immobilized ligand;
 - (b) separating any unbound carbohydrate-free transferrin from any bound carbohydrate-containing moieties;
 - (c) contacting any separated carbohydrate-free transferrin with an anti-transferrin antibody or an anti-transferrin antibody fragment to form a conjugate; and
 - (d) detecting the presence of any carbohydrate-free transferrin antitransferrin antibody conjugate by tubidometry or nephalometry.
- 14. (New) The method of claim 13, wherein the presence of any carbohydratefree transferrin is indicative of alcohol abuse.
- 15. (New) The method of claim 13, wherein the method is free from the influence of amino acid sequence polymorphism in the polypeptide backbone of an abuser's transferrin.
- 16. (New) The method of claim 13, wherein the method is independent of the abuser's race.
- 17. (New) A kit for use in a method of claim 13, the kit comprising:
 one or more carbohydrate-binding ligands;
 means for separating unbound carbohydrate-free transferrin from bound
 carbohydrate-containing transferrin; and

